

Project Overview

What's in the Smoke from Prescribed Fire?

Project Context & Background

The goal of this project is to look for polycyclic aromatic hydrocarbons (PAHs) in fine particulate matter generated during prescribed burns of the Coconino National Forest in northern Arizona.

Scientists are interested in the organic composition of PM-2.5 because of its potential impact on health, climate, and visibility.

To our knowledge, this work represents the first effort to examine PAH concentrations in PM-2.5 generated during prescribed fire, although other studies have analyzed fine particle emissions from foliar fuel combustion and residential wood burning.

Who is This Project For?

This is an excellent project for chemistry majors interested in environmental, analytical, or atmospheric chemistry, as well as students who want to learn more about gas chromatography/ mass spectrometry (GC/MS) as an analytical tool.

What will I do?

In this project, you will analyze PAH concentrations in PM-2.5 from three different prescribed burns using GC/MS. Prescribed burns are analyzed during the ignition and smoldering phases.



Before (above) and after (below) prescribed fire



Canned Research Project

What Will I Learn About?

- Role of prescribed fire in forest restoration
- PM-2.5 and PAHs – what they are and why they're important
- Soxhlet extraction and Kuderna-Danish concentration
- Use of surrogates & internal standards in GC/MS

Note: Some familiarity with Excel is required

To Learn More

- Fine, P.M.; Cass, G.R.; Simoneit, B.R.T. Chemical Characterization of Fine Particle Emissions from Fireplace Combustion of Woods Grown in the Northeastern U.S. *Environ. Sci. Technol.* **2001**, *35*, 2665-2675.
- Fine, P.M.; Cass, G.R.; Simoneit, B.R.T. Chemical Characterization of Fine Particle Emissions from Fireplace Combustion of Woods Types Grown in the Midwestern and Western United States. *Environ. Engin. Sci.* **2004**, *21*, 387-409.
- Hays, M.D.; Geron, C.D.; Linna, K.J.; Smith, N.D.; Schauer, J.J. Speciation of Gas-Phase and Fine Particle Emissions from Burning of Foliar Fuels. **2002**, *36*, 2281-2295.
- Malm, W.C.; Day, D.E. Optical Properties of Aerosols at Grand Canyon National Park. *Atmos. Environ.* **2000**, *34*, 3373-3391. (*Discusses how particles cause haze*)
- Oanh, N.T.K.; Nghiem, J.H.; Phyu, Y.L. Emission of Polycyclic Aromatic Hydrocarbons, Toxicity, and Mutagenicity from Domestic Cooking using Sawdust Briquettes, Wood, and Kerosene. *Environ. Sci. Technol.* **2002**, *36*, 833-839.
- Schauer, J.J.; Kleeman, M.J.; Cass, G.R.; Simoneit, B.R.T. Measurement of Emissions from Air Pollution Sources. 3. C1-C29 Organic Compounds from Fireplace Combustion of Wood. *Environ. Sci. Technol.* **2001**, *35*, 1716-1728.
- Schwartz, S. E.; Buseck, P. R. Absorbing Phenomena. *Science* **2000**, *288*, 989-990. (*Discusses how particles affect climate*)



Polycyclic Aromatic Hydrocarbons in Prescribed Fire

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This is a two-part description of your canned research project. The first part (Cover) is a somewhat flashy introduction to the project, highlighting features of the project and listing readings in the research area. The second part (Project Overview) places the work in context, emphasizing why the work is important and providing an overview of the project's purpose and methods.	
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This section describes work that has already been completed by previous members of your research group. Your project begins where their work ended. You can “cut and paste” sections of this text into the Methods section of your paper; hence, we have also included parts of it in the Word document PAH Previous Work File.doc .	
Methods	5
This section describes, step-by-step, the procedures that you will follow in conducting the canned research. It is written like a lab manual or lab notebook. Your goal will be to rewrite these procedures (combined with the previous work described above) in your Methods section, in a style appropriate for a journal article.	
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This section shows you how to analyze your data and refers you to the Excel files that contain the data (PAH Project CalData.xls and PAH Project Data.xls). Most of this work will not get reported in your final paper; however, it must be completed in order to arrive at your results.	
Results	15
This section prompts you to consider important features of your results, as well as ways to present your data to an expert audience. This section is presented largely as a series of questions for you to consider. You will ultimately present your findings in your Results section using both text and one or more graphics (e.g., table or figure).	
Discussion	15
This section prompts you to consider what your results mean, why they are important, and how they may apply to a larger context. This section is presented largely as a series of questions for you to consider. You will present the interpretations and implications of your research in your Discussion section.	

Project Overview

The fire-suppression policies of the past century have left national forests in an unhealthy state characterized by dense undergrowth and heavy fuel loads, conditions that have increased the likelihood of catastrophic wildfire. To reduce this threat, Federal, tribal, and State land managers have agreed to increase their use of prescribed (or intentional) fire, to restore wildland ecosystems to their pre-1900s condition. Along with the benefits of prescribed fire, however, are risks associated with fire-generated pollutants. Foremost among these is an expected increase in fine particulate matter or PM-2.5, airborne particles with aerodynamic diameters of $\leq 2.5 \mu\text{m}$. Such particles are small enough to reach the alveoli in the lungs and have been linked to pulmonary disease, morbidity, and even death.

The health effects of PM-2.5 are related not only to their size but also to their composition. For example, PM-2.5 generated during residential wood burning (RWB) of both hard and soft woods has been shown to contain polycyclic aromatic hydrocarbons (PAHs), species known for their mutagenic and carcinogenic effects. The PAH levels in wood smoke from prescribed fire, however, are less well understood. Prescribed fire is different in several ways from RWB; most notably, prescribed fire consumes primarily pine needles and small diameter branches, not logs. Logs are more resinous than needles, which can result in higher PAH production. Moreover, prescribed fires are low-intensity burns with both smoldering and flaming stages; smoldering favors PAH production, while flaming favors more complete combustion into volatile organics, carbon dioxide, and water. Because of these differences, it is important to examine the PAH content of wood smoke generated during prescribed fire.

Statement of Purpose: The goal of this canned research project is to analyze PAH concentrations in PM-2.5 generated during prescribed fires of the Coconino National Forest near Flagstaff, AZ. The Coconino National Forest is the largest contiguous ponderosa pine forest in North America and is scheduled for intensive restoration efforts through prescribed fire in the next decade. Few studies exist on PAH levels in wood smoke from prescribed fire, and this is the first study to analyze wood smoke during prescribed burns of the Coconino National Forest.

Method Overview. In this project, you will analyze the PAH content in PM-2.5 collected during prescribed fires of the Coconino National Forest. PM-2.5 will be extracted into an organic solvent (dichloromethane) and concentrated to 1 mL. PAH levels in the extract will be analyzed using GC/MS. You will compare the chromatographs and spectra of your samples with selected PAH calibration standards. PAH concentrations will be reported as a mass fraction (μg PAH per mg particulate).

Previous Work

Research projects are long-term and involve many different investigators over several semesters or years. Your "canned" research project is no exception, and you will be joining the project midstream. Previous members of your group have already collected, weighed, extracted, and concentrated the fire-generated PM-2.5 samples. The 1 mL extracts were stored in the freezer, waiting for you to analyze them by GC/MS. Thankfully, these students wrote up their methods section before they left (Excerpt 1). Additional information is included in their lab notebook (Excerpt 2). You may copy the information in Excerpt 1 and include it in the experimental section of your journal article. Yes, that's right, you can *copy* it, but only on the condition that you *read and analyze* it and use it as a guide for writing your part of the experimental section. (For this purpose, it is included in the Word document **PAH Previous Work.doc.**) Copying a pre-written methods section may seem unusual, but in fact, this process is common in research. Research groups often describe the same experimental techniques in multiple papers; hence, they use the same description in each paper. The first paper describes the methods in the most detail; subsequent papers are briefer and cite the first paper for readers who would like more information.

Excerpt 1. (What Previous Members of Your Group Did)

Experimental Section

Prescribed Burns. PM-2.5 was collected during three prescribed burns conducted by the Peaks Ranger District of the Coconino National Forest Service in October 2003. The prescribed burns took place on three adjacent sites (A-C) of the A1-Mountain region of the Coconino National Forest, a predominantly ponderosa pine (*Pinus ponderosa*) forest near Flagstaff, AZ. All fires were broadcast burns and consumed ~100 acres of downed woody material, litter (dead-brown pine needles), and duff (decomposing litter). Each prescribed burn was sampled during two fire events, ignition/flaming (day 1) and smoldering (day 2).

PM-2.5 Collection. During each fire event, ambient air was sampled using a battery-operated PM-2.5 chemical speciation monitor, equipped with a sharp-cut cyclone to remove particles with aerodynamic diameters $>2.5 \mu\text{m}$ (MetOne SuperSASS, Grants Pass, Oregon). Typical flow rates were 6.5 lpm. Sampling times were 2 h. All samples were collected on Teflon filters (47 mm), which had been pre-conditioned in a control chamber (24 h, 20-25 °C, and 30-40% relative humidity) and pre-weighed (Mettler XS105 analytical balance, $\pm 0.02 \text{ mg}$) for particulate mass analysis. Two samples were collected simultaneously. One sample was analyzed for toxicity (described elsewhere); the other was analyzed for PAH concentrations (this work). One field blank and one lab blank were analyzed for each fire event.

Extraction and Concentration. Filters (6 exposed filters, 6 field blanks, and 3 lab blanks) were extracted in dichloromethane (DCM) using a Soxhlet apparatus and concentrated to 1 mL in a Kuderna-Danish concentrator according to EPA Method 3540C. Prior to extraction, each filter was spiked with 50 μL nitrobenzene- d_5 (64 mg/L) to measure percent recovery. Extracts were stored in precleaned amber glass bottles at -20 °C until analyzed.

Excerpt 2. Lab notebook summarizing PM-2.5 filter data

October 30, 2003

Filter log for filters used in PAH analysis.

Three prescribed fires (RX1-RX3) were conducted on three adjacent plots (A-C) of the A1-Mountain region of the Coconino National Forest. Sampling time was 2 hours per event. Masses are the average of three weighings (± 0.02 mg).

BDL indicates masses ≤ 0.02 mg

Sample No.	Fire/Area	Date	Event	PM-2.5 Mass (mg)
S1_IG/F	RX1 (A)	10/13/03	Ignition/flaming	2.01
FB1_IG/F	RX1 (A)	10/13/03	Ignition/flaming	BDL
S1_SM	RX1 (A)	10/14/03	Smoldering	1.54
FB1_SM	RX1 (A)	10/14/03	Smoldering	BDL
S2_IG/F	RX2 (B)	10/22/03	Ignition/flaming	2.38
FB2_IG/F	RX2 (B)	10/22/03	Ignition/flaming	BDL
S2_SM	RX2 (B)	10/23/03	Smoldering	1.62
FB2_SM	RX2 (B)	10/23/03	Smoldering	BDL
S3_IG/F	RX3 (C)	10/27/03	Ignition/flaming	3.2
FB3_IG/F	RX3 (C)	10/27/03	Ignition/flaming	BDL
S3_SM	RX3 (C)	10/28/03	Smoldering	1.58
FB3_SM	RX3 (C)	10/28/03	Smoldering	BDL

Research Steps

Research projects are complex and this Canned Research is no exception. It is often useful to divide such a large project into smaller tasks. **Research Steps** will help you with this task. Unless assigned by your instructor, these steps are not required; however, by following these steps, you will more closely imitate the research process.

At this point in your project, it is important that you understand what the previous members of your group accomplished.

- Read through Excerpt 1. Make a list of any terms or methods that you do not understand. Research these items (using textbooks, the internet, journal articles) to find out what they mean and how they are used.
- Find two or more peer-reviewed journal articles that describe procedures similar to those used in Excerpt 1. Read the Methods section of these articles to better understand the procedures and to find models for the ways experts write about these procedures. Based on these journal

articles, reread Excerpt 1. Decide if there are any corrections, revisions, or changes you would like to make before you include it in your paper.

- **Consider Collaboration.** According to Excerpt 1, two PM-2.5 samples were collected simultaneously during each fire event. One sample was used to test for toxicity, the other to measure PAH levels. This project pertains only to PAH analysis; a separate canned research project (*Toxicity of Prescribed Fire*) addresses the toxicity issue. Because PAHs may contribute to toxicity, you might want to collaborate with students in your class that have selected the toxicity project, or ask your instructor for information about the toxicity study.

Methods

The methods for your canned research project are described below. Note the change in format. The Previous Work section was written as it might appear in a journal article. The information below is written as it might appear in a lab manual or lab notebook. Your goal will be to rewrite this information for an expert audience in a journal article format.

Notes on terminology and abbreviations

Polycyclic aromatic hydrocarbons (PAHs). We are looking for 8 specific PAHs in our PM-2.5 extracts

Analyte. What you analyze your samples to find (in our case, the 8 PAHs)

Calibration standards (CS). The GC/MS must be “calibrated” for each analyte you expect to see in your study. To do this, we purchased a mixture of 8 PAHs of known concentrations. From this, we will prepare 5 calibration standards, varying from least to most concentrated. We will use these standards to plot a calibration curve.

Surrogate Standards (SS) (also called a recovery standard). The filters that contain the PM-2.5 (after PM-2.5 collection, but before extraction and concentration) are “spiked” with a known amount of one or more surrogate standard. Often the SS is deuterated (D instead of H), so that you know that your spike is the only source of this compound in your sample. After spiking, the filter is extracted and concentrated as usual. The GC/MS analysis shows how much SS made it through extraction, concentration, and the GC column. We will use this information to estimate the recovery of our PAHs. Our SS is nitrobenzene-d5.

Internal Standard (IS). A known amount of internal standard (also known as a reference standard) is added to all samples (calibrations standards and PM-2.5 extracts) just before injection into the GC/MS. PAH peaks will be quantified relative to the internal standard peak in each run. Our internal standard will be 1,4-dichlorobenzene.

I. Prepare stock solutions for calibration standards, internal standard, and surrogate standard

Reagents (VWR, research grade, unless otherwise stated)

Dichloromethane (DCM) (solvent, residue grade)

PAH mixture (**CS**, 8 PAHs, MW 128-202 amu, 2000 µg/mL in 1 mL DCM)
(UltraScientific) (see *Table 1 for list of PAHs*)

1,4 Dichlorobenzene-d4 (**IS**) (2000 mg/L in 1 mL DCM) (UltraScientific)

Nitrobenzene-d5 (**SS**) (2000 mg/L in 1 mL DCM) (UltraScientific)

Equipment

- variable volume micropipettes (10-100, 100-1000 μ L)
- 1 mL glass pipette, precleaned and baked
- volumetric flasks (5, 10, 25, 100 mL), precleaned and baked
- precleaned (PC) amber bottles with Teflon caps (10, 20, 25, 40, 125 mL)

Procedure

- Prepare CS stock solution (64 mg/L): Take 0.80 mL of PAH mixture (2000 mg/L) and dilute to 25 mL in DCM. Store in 40 mL amber bottle.
- Prepare IS stock solution (64 mg/L): Take 0.80 mL of 1,4 dichlorobenzene-d4 (2000 mg/L) and dilute to 25 mL in DCM. Store in 40 mL amber bottle.
- Prepare SS stock solution (64 mg/L): Take 0.80 mL of nitrobenzene-d5 (2000 mg/L) and dilute to 25 mL in DCM. Store in 40 mL amber bottle.

II. Prepare calibration standards (CS1-CS5) and lab blanks (LB1-LB3)

Using the stock solutions, prepare a 5 mL volume (final volume 5.2 mL) of 5 calibrations standards (CS1-CS5). Each standard will contain 8 PAHs and the SS, in equal concentrations, varying from the lowest concentration (CS1=1.231 mg/L) to the highest (CS5=3.692 mg/L).

200 μ L of IS stock (@64 mg/L) will be added in a separate step, resulting in a final volume of 5.2 mL. (The concentrations shown below for CS1-CS2 are based on the final 5.2 mL volume.) The final IS concentration in CS1-CS5 will be 2.462 mg/L (across all 5 standards).

Lab blanks (LB1, LB2, LB3) will contain no PAH or SS, but will have the IS added in a later step; the final concentration of the IS in LB1-LB3 will be 2.462 mg/L.

Procedure

- **LB1 (LB2, LB3)(0 mg/L):** 5 mL DCM. Store in freezer in 10 mL glass bottle.
- **CS1 (1.231 mg/L):** Mix 100 μ L PAH stock (@64 mg/L) and 100 μ L SS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer in 10 mL glass bottle.
- **CS2 (1.846 mg/L):** Mix 150 μ L PAH stock (@64 mg/L) and 150 μ L SS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer in 10 mL glass bottle.
- **CS3 (2.462 mg/L):** Mix 200 μ L PAH stock (@64 mg/L) and 200 μ L SS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer in 10 mL glass bottle.
- **CS4 (3.077 mg/L):** Mix 250 μ L PAH stock (@64 mg/L) and 250 μ L SS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer in 10 mL glass bottle. .
- **CS5 (3.692 mg/L):** Mix 300 μ L PAH stock (@64 mg/L) and 300 μ L SS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer in 10 mL glass bottle. .

III. Obtain 5 point calibration curve for each PAH and the SS

Instrumentation

- The GC/MS instrument you will use is a Hewlett Packard 5890 Series II with 5971A mass selective detector with enhanced ChemStation V. A03.00 software. The instrument will be operated in selected ion mode, using splitless injection with a DB-5 capillary GC column (30 m length, 0.25 mm id, and 0.25 μ m film thickness).
- Program the GC oven temperature as follows: 40 $^{\circ}$ C (5 min), ramp to 300 $^{\circ}$ C at 10 $^{\circ}$ C per minute.

Procedure

- Step 1.** Do a background run. Be sure chromatograph looks clean with no evidence of contamination. Bake column overnight if needed.
- Step 2.** Run a solvent blank. Inject a 2 μL sample of DCM (with solvent override enabled). Chromatograph should look “clean” with no evidence of contamination. (Hint: Use a pre-cooled syringe and store vials on ice to prevent evaporation.)
- Step 3.** Add 200 μL of IS stock (@64 mg/L) to each calibration standard, bringing the total volume to 5.2 mL. The IS concentration in all 5 calibration standards will be 2.462 mg/L.
- Step 4.** Inject 2 μL of each calibration standard, beginning with the lowest concentration (CS1) and ending with the highest concentration (CS5). (Hint: Use a pre-cooled syringe and store vials on ice to prevent evaporation of DCM.)
- Step 5.** Collect a chromatograph for each run. Each chromatograph will have 10 peaks (IS, SS, 8 PAHs). You will be able to distinguish the 10 compounds based on their retention times and major fragment ions (Table 1).

Table 1. Retention times, molecular weights, and major fragment ions for PAHs.

Label	PAH	Ret Time (min)	MW	Major Ion (m/z)
IS	1,4-dichlorobenzene-d ₄	12.89	147.00	152
SS	nitrobenzene-d ₅	15.05	128.07	128
PAH-1	naphthalene	17.82	128.17	128
PAH-2	acenaphthylene	24.36	152.20	152
PAH-3	acenaphthene	25.15	154.21	154
PAH-4	fluorene	27.29	166.22	166
PAH-5	phenanthrene	31.26	178.23	178
PAH-6	anthracene	31.45	178.23	178
PAH-7	fluoranthene	36.26	202.26	202
PAH-8	pyrene	37.15	202.26	202

Research Steps

It is important that you understand the methods that are being used in your canned research. Until you understand the instruments and procedures, it will be very difficult to understand the data.

- Read through the procedures and instrumentation described above, and make a list of any terms, procedures, or instrumentation that you are not familiar with. Research these terms/procedures /instruments using any resources available (e.g., textbooks, the internet, journal articles).
- Be sure that you understand the following terms, including why they are important for your project :

internal standard	calibration standard
laboratory blank	analyte
calibration curve	surrogate standard

- Find two or more peer-reviewed journal articles that describe procedures that are similar to those that used in your Canned Research. Reading the Methods section of these articles may help you understand the procedures better and will model the way experts write about these procedures for an expert audience. Based on these journal articles, read through the methods described above and jot down any additional questions/terms you have. Research the answers to your questions.



Before proceeding with your Methods, it is wise to check the calibration data you've just collected. If you simply assume that all is well, you may delay catching a problem either with your laboratory method, the instrument performance, or in your data analysis procedures.

Go to the Calibration Curve subheading of the Data Analysis section (page 10) and learn how to analyze your calibration data.

IV. Run PM-2.5 samples, lab blanks, and field blanks

You are now ready to examine the PAH content in the fire-generated PM-2.5 extracts, as well as the quality control samples (lab and field blanks). All PM-2.5 extracts (1.00 mL) have been stored in the freezer.

- Step 1.** Prior to injection, add 50 μL of IS stock (@64 mg/L) to each vial, bringing the total volume to 1.05 mL. (*The final concentration of the IS will be 3.048 mg/L in the PM-2.5 samples.*)
- Step 2.** Inject 1 μL of each sample to obtain the chromatograph. (Be sure to pre-cool each syringe and vial, as described above.) You will run extracts from the exposed filters in triplicate, injecting 3 x 1 μL samples from each vial. You will run field and lab blank extracts only once, totaling 27 runs (3 x 6 exposed filters, 1 x 6 field blanks, and 1 x 3 lab blanks).



You've now completed your methods, so you need to analyze your authentic sample data before you can move on to the Results section.

Go to the Authentic Samples subheading of the Data Analysis section (page 11) and learn how to analyze your PM-2.5 extracts for PAH content.

Data Analysis

Data analysis (calculations) is an important step in conducting research, a step that links the raw data collected to the final meaningful results that are presented in journal articles. This section will guide you through the following steps in analyzing your data:

- I. Preparing a calibration curve for the PAHs
- II. Processing data from the prescribed fire (Rx fire)
 - A. Calculating PAH concentrations in the prescribed fire sample extracts
 - B. Converting extract concentrations ($\mu\text{g/mL}$) to mass fractions ($\mu\text{g/mg}$)
 - C. Calculating mean mass fractions and standard deviations for authentic samples

Research Steps

It is important that you understand the calculations that lead to the final results presented in your paper.

- Read through the procedures/equations described in each section below, and make a list of questions you may have. Research these questions using any resources available.
- Often students first attempt to use calculators to analyze their data. We discourage this approach and recommend that you use a spreadsheet program (Excel) instead. Although the calculator-approach may seem easier at first, in the long run calculators are time-consuming and error-prone. For example, jotting down numbers and using a calculator for multi-step calculations often leads to rounding errors. Moreover, calculations “by hand” do not provide a track record of your steps. By using a spreadsheet, you have an electronic record of your calculations; the spreadsheet is easily corrected and can serve as a permanent record of your data analysis.
- This canned research project assumes that you have already used Excel and are familiar with using formulas and preparing graphs. If you are a beginner, try using the built-in Excel help or ask a friend or instructor to walk you through the spreadsheets. It will not take long before you realize how much easier it is to use Excel than a calculator!

A. Calibration Curve

You will use the peak areas in the chromatographs to plot a 5-point calibration curve for each PAH (8 graphs) and the SS (1 graph). The calibration data are included in the Excel file entitled **PAH CalData.xls**. This file is divided into three worksheets. The worksheets are labeled by tabs at the bottom of the Excel page (Calibration Data, Sample Calibrations, Remaining Calibrations). Clicking on a tab will move you back and forth between the worksheets.

Here, we show you how to analyze the data in the worksheets to obtain a calibration curve for each PAH and the SS. For more information on the formulas used to obtain these calibration curves, see Section C (Understanding Calibration Curves).

1. Click on the first tab (Calibration Data). Here you will see the chromatograph data for the runs of the 5 calibration standards.
2. Click on the second tab (Sample Calibrations). This worksheet shows you how to use the chromatograph data to plot calibration curves for PAHs 1-4. The data are arranged in 6 columns:

Run	1-5, corresponding to the 5 calibration runs
PAH area	from chromatograph, copied and pasted from Calibration Data
[IS] mg/L	the concentration of the IS (a constant)
IS area	from chromatograph, copied and pasted from Calibration Data
X	a formula which calculates the values for the x-axis (to see the formula click on cell 7E, highlighted in yellow).
Y	the values for the y-axis, which are the concentrations of the PAH in the 5 runs (copied and pasted from the Calibration Data)

Following the columns of data, you will see a scatter graph of the XY data, plotted using Excel. A linear curve (trendline) was fit to the data. The equation for the line and the R-squared value are shown on the graph. ***You will need this equation to analyze your authentic samples.***

Your Turn. Click on the third tab (Remaining Calibrations). This worksheet has been set up for you to create the 5 remaining calibration curves (PAHs 5-8 and SS). To make things easier, we have transposed all of the column data except for the PAH areas and the formula to calculate values for the x-axis (both areas are highlighted in yellow). Complete these cells and then plot the curves, fitting each curve with a trendline. (If you are a beginner, you may need to seek help in using Excel).

B. Authentic Samples

The GC/MS analysis of the authentic samples will produce chromatographs that you will use to determine PAH concentrations. The data are included in the Excel file entitled **PAH Data.xls**. This file is divided into 7 worksheets. The worksheets are labeled by tabs at the bottom of the Excel page (Field Blanks, Lab Blanks, Rx1, Rx2, Rx3, Sample PAH Calculation, Sample Percent Recovery). Clicking on a tab will move you back and forth between the worksheets. Here, we show you how to use these worksheets to determine the concentration of the targeted PAHs in your samples.

1. Click on the first tab (Field Blanks). Examine the field blank data. Was there any evidence of field contamination? Why or why not?
2. Click on the second tab (Lab Blanks). Examine the lab blank data. Was there any evidence of lab contamination? Why or why not?
3. Click on tabs 3, 4, and 5 (Rx1, Rx2, and Rx3). These worksheets contain the GC/MS data from the filter extracts from each fire event (separated into ignition and smoldering). Look to see which PAHs were above the detection limit. (We only need to calculate concentrations for PAHs that were above the detection limit in any given fire event).
4. Click on tab 6 (Sample PAH Calculation). We have completed the calculation for PAH-7 (fluoranthene). Note that the worksheet is set-up to calculate PAH-7 levels in all three burns, under both ignition and smoldering conditions. The first 5 columns are data needed from other worksheets. For example, for Rx1 we need

Area(PAH-7)	data from Rx1 (Ignition, runs 1-3; Smoldering, runs 1-3)
Area(IS)	data from Rx1 (Ignition, runs 1-3; Smoldering, runs 1-3)
[IS] mg/L	concentration of internal standard (a constant)
RF	response factor (slope from calibration curve for PAH-7)
b	noise (intercept from calibration curve for PAH-7)

Column 6 is a formula that calculates the concentration (mg/L= μ g/mL) of PAH-7 in the 1 μ L sample injected into the GC/MS. The formula is based on the following equation:

$$[\text{PAH-n}] = \text{RF} \times \frac{\text{Peak Area}_{\text{PAH-n}} \times [\text{IS}]}{\text{Peak Area}_{\text{IS}}} + \text{noise} \quad 1$$

where

[PAH-n]=	conc. (mg/L= μ g/mL) of PAH-n in sample (for n = 1, 2, 3....8)
RF=	response factor (m=slope) from calibration curve (unitless)
Peak Area _{PAH-n} =	peak area from chromatograph for PAH-n
[IS]=	conc. of internal standard in each sample (3.048 mg/L)
Peak Area _{IS} =	peak area from chromatograph for IS
noise=	y-intercept (b) from calibration curve (mg/L)

Column 7 is a formula to calculate the mass of PAH-7 (in μ g) in 1.05 mL (the extracted volume per filter), based on the concentration of PAH-7 (column 6).

Columns 8 and 9 convert the mass of PAH-7 in the extracted volume to a mass fraction (the desired units) by dividing the PAH-7 mass (μ g, column 7) by the PM-2.5 mass (mg, column 8): μ g PAH-7/mg PM-2.5. These units are equivalent to "parts per thousand", where 1 μ g/mg = 1 ppt = 0.10%. This allows PM-2.5 collected under different burn conditions (ignition and

smoldering) and emission levels (higher during ignition) to be compared, because the concentrations are normalized to a "per milligram" PM-2.5 basis.

Columns 10 and 11 calculate the average and standard deviation for the 3 runs.

The calculations are repeated for all three prescribed burns. The data for PAH-7 are summarized in a graph at the bottom of the page.

Your Turn. Using the PAH-7 worksheet for guidance, calculate the mass fraction of PAH-8 in $\mu\text{g}/\text{mg}$. (Hint: You might want to copy the worksheet and paste it into a new worksheet or file.)

5. Click on tab 7 (Sample Percent Recovery). Recall, prior to extraction and concentration, each filter was spiked with 50 μL nitrobenzene- d_5 (@64 mg/L), the surrogate standard. This was extracted into a final volume of 1.05 mL; hence, the final concentration of SS (assuming 100% recovery) should be

$$50 \mu\text{L} * (1 \text{ mL}/1000 \mu\text{L}) * (64 \text{ mg/L}) * (1/1.05 \text{ mL}) = 3.05 \text{ mg/L} \quad 2$$

As shown in columns 1-6, the recovered concentration of SS in each run is determined the same way you calculated PAH concentrations above.

To determine percent recovery, divide the recovered SS concentration (column 6) by the original SS concentration (3.05 mg/L in column 7). After multiplying by 100%, the answer is shown in Column 8.

$$\text{Percent Recovery} = \frac{\text{Recovered SS Conc. (mg/L)}}{\text{Original SS Conc. (3.05 mg/L)}} \times 100\% \quad 3$$

On Your Own. Repeat this calculation for prescribed fires Rx2 and Rx3. Determine (and report) the average percent recovery (and standard deviation) over all 3 fires (18 measurements).

C. Understanding Calibration Curves

Within a range of concentrations (denoted as the linear range), the concentration of PAHs (or SS) will be proportional to their integrated peak area (eq 4). Similarly, the concentration of the internal standard (IS) is proportional to its integrated peak area (eq 5).

$$[\text{PAH-1}] \propto \text{Peak Area}_{\text{PAH-1}} \quad 4$$

$$[\text{IS}] \propto \text{Peak Area}_{\text{IS}} \quad 5$$

Due to instrumental variability, taking a ratio of areas is more reproducible using absolute areas of individual compounds. One of the causes of this is that not ALL of the analyte molecules are properly vaporized in the injector, carried down the GC column, and properly detected. The % of molecules actually detected is very sensitive to changes in temperature, pressure, flowrate, and

the electronics in the detector. For example, let's take the following hypothetical duplicate runs (same sample run twice).

Run 1: Peak Area $_{PAH-1}$ = 333,000
 Run 2: Peak Area $_{PAH-1}$ = 487,003 counts
 A change of ~32%!

If, however, we take the ratio of PAH peak area (or SS area) to the IS peak area, for which the concentration never changes (~3.048 ug/L in our case), we have the following hypothetical scenario:

Run 1: Peak Area $_{PAH-1}$ = 333,000 counts Peak Area $_{IS}$ = 1,250,000 counts
 Run 2: Peak Area $_{PAH-1}$ = 487,003 counts Peak Area $_{IS}$ = 1,828,089 counts

For whatever reason, Run 1 detected a smaller fraction of molecules. If we assume that because the PAH/SS of interest and our IS are similar in molecular weight and chemical structure, they behave similarly in response to any variable conditions. Thus, when fewer PAH/SS molecules are detected, so are fewer IS molecules. When we take the ratios for a given run . . .

$$\begin{array}{l} \text{Run \#1} \quad \frac{\text{Peak Area}_{PAH-1}}{\text{Peak Area}_{IS}} = \frac{333,000}{1,250,000} = 0.2664 \\ \text{Run \#2} \quad \frac{\text{Peak Area}_{PAH-1}}{\text{Peak Area}_{IS}} = \frac{487,003}{1,828,089} = 0.2664 \end{array}$$

The same ratio!! Thus, the use of an internal standard minimizes the effect of instrument variability from run-to-run. Thus, for our calibration curve, we use this ratio, rather than just the peak area of the PAH. We can say the ratio of a given PAH and IS concentrations is proportional to their peak area ratio. Mathematically,

$$\frac{[PAH-1]}{[IS]} \propto \frac{\text{Peak Area}_{PAH-1}}{\text{Peak Area}_{IS}} \quad 6$$

Eq. 6 can be rearranged to solve for the concentration of PAH-1 (eq 7).

$$[PAH-1] \propto \frac{\text{Peak Area}_{PAH-1}}{\text{Peak Area}_{IS}} \times [IS] \quad 7$$

And the proportionality can be converted into an equality through the use of a proportionality constant, the response factor (RF) (Eqn. 8)

$$[PAH-1] = RF \times \frac{\text{Peak Area}_{PAH-1}}{\text{Peak Area}_{IS}} \times [IS] \quad 8$$

Equation 8 is the equation of a line ($y = mx + b$), where RF is the slope (m). RF can be determined by plotting y (the known PAH/SS cal. Std. concentrations) versus x (the ratio of PAH/SS and IS peak areas multiplied by the known IS concentration). The intercept b should equal zero, corresponding to $[PAH] = 0$; however, typically a non-zero value is found representative of background noise in GC/MS.

$$y = m \times x + b$$

$$[\text{PAH-1}] = \text{RF} \times \frac{\text{Peak Area}_{\text{PAH-1}} \times [\text{IS}]}{\text{Peak Area}_{\text{IS}}} + \text{noise}$$

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Once a linear equation is established (i.e., RF and b are obtained from the best fit line to the calibration standards) for EACH PAH and the SS, you have a complete set of calibration curves for all the compounds you wish to quantify. In other words, you will make one curve for each PAH compound and an additional curve for the SS.

Results

Once the numbers have been crunched, it is time to think about your data. Use the following questions to guide your presentation of the data.

- What story do the data tell about PAH levels in wood smoke from prescribed fires of ponderosa pine? What will be the “take-home” message you want to make? How can you present the data (consider tables, figures, and text) to emphasize that take-home message?
- There are several different ways that you can organize your results. One way is by fire event (Rx 1, Rx 2, and Rx 3), another is by fire phase (ignition/flaming or smoldering), and a third is by PAH. Think about these different ways and decide which presentation is best for your paper. *You should limit yourself to no more than two graphics (tables, figures) in your paper.*

Discussion

How will you discuss your results? Consider the following guidelines as you write the discussion of your paper.

- Were the data consistent? If so, what trends can you discern? Highlight these in your discussion.
- Consider why some PAHs may be present in greater abundance in wood smoke than others (hint: consider their molecular weight and volatility; PAHs that are too volatile will evaporate and will not stay condensed on the particulate).
- If you collaborated with a student who wrote about the toxicity in wood smoke, what insights (if any) did their data suggest?
- How did the data compare to literature values? What might be some reasons for the similarities and differences you observed? How relevant do you think these results will be to other types of wood (e.g., hardwoods) or other types of fire (wildfires, catastrophic fires, residential wood-burning, etc.)?
- How do you think the information obtained in your study may be important in a larger context. What might be the implications or applications of your findings?

**REMEMBER THESE DATA,
ALTHOUGH BASED ON PRELIMINARY RESULTS,
ARE FICTITIOUS!
HAVE FUN WITH YOUR INTERPRETATIONS
BUT DON'T TAKE THEM TOO SERIOUSLY!**